Use of Defined Competitive Exclusion Cultures to Enhance Colonization Resistance to Enteric Pathogens

D.J. Nisbet[1], D.E. Corrier[1], P. J. Fedorka-Cray[2], and J.R. DeLoach[1]
[1]USDA/ARS, Food Animal Protection Research Laboratory
College Station, Texas 77845 and [2]USDA/ARS, National Animal
Disease Center, Ames, IA 50010

During the past several years our laboratory has conducted research towards developing defined competitive exclusion cultures that enhance colonization resistance against salmonellae in baby chicks. Previously in our laboratory it was shown that 10-day-old broiler and layer chicks that were fed diets containing 5-10% lactose provided either in the feed or in water from day-of-hatch were significantly more resistant to *Salmonella typhimurium*, and *S. enteritidis* cecal colonization than control chicks not provided a diet supplemented with lactose. Additionally, resistance against salmonellae cecal colonization was further enhanced in treatment groups provided dietary lactose in combination with an undefined mixture of anaerobic bacteria (i.e. undefined competitive exclusion culture or Nurmi culture) originally obtained from the ceca of adult broiler chickens maintained on a diet containing lactose. In order to make a defined competitive exclusion culture that was efficacious in enhancing colonization resistance against salmonellae, we cultured cecal contents obtained from adult broilers maintained on a unmedicated diet containing 5% lactose in a continuous-flow (CF) culture apparatus (i.e. chemostat), that was maintained at parameters that would best represent the cecal environment. Two different CF-cultures were developed from the same initial inoculum with the difference being that one CF-culture medium contained lactose and the other did not. From these CF cultures two different defined cultures were developed (CF1and CF2) that were efficacious in enhancing colonization resistance against *S. typhimurium* in broilers and *S. enteritidis* in layers, when used in combination with diets containing lactose. Although both of these CF cultures significantly protected day-old chicks against salmonellae colonization production economics dictated that a defined culture be developed that was efficacious in controlling salmonellae cecal colonization in the absence of dietary lactose. A third CF-culture was developed employing the same techniques used for the previous CF-cultures with notable exceptions. The original inoculum came from adult broilers fed an unmedicated diet that did not have lactose included in the formulation, and the culture medium also had no lactose. From this inoculum a CF-culture containing 29 different bacterial isolates was developed (CF3). This CF-culture has been shown under experimental conditions to significantly protect against *S. typhimurium* and *S. enteritidis* colonization and in a large commercial field trial to significantly reduce salmonellae colonization in adult market-age broilers. Other research with CF3 has shown that chicks provided the culture at day-of-hatch have greater than a 100-fold increase in cecal anaerobic CFU at three-days-of-age compared to untreated controls. Additionally it has been shown by electron microscopy that a large proportion of this increase occurs on the cecal mucosal epithelium. The results of this rapid establishment of a cecal bacterial ecosystem is a significant increase in cecal total volatile fatty acids, with the most notable increase in propionic acid. Over several studies the correlation between the level of cecal propionic acid in three-day-old chicks and salmonellae colonization has been extremely high and we now use this physiological response as a indicator of CF3 culture establishment and a predictor of efficacy in laboratory experiments. This increase in propionic acid was also observed in the commercial field trial mentioned previously. This technology has been licensed to an industry partner whom we have a cooperative research and development agreement (CRADA) with and is currently in the FDA process and a large scale commercial fermentation facility is under construction. Currently we are employing the same techniques used to develop CF1, CF2, and CF3 to develop a defined CF-culture that will be

efficacious in reducing salmonellae colonization in swine. This research is being conducted under a CRADA with our industry partners and in cooperation with the USDA/ARS Salmonellosis in Swine research unit at Ames Iowa. Presently a CF- culture has been developed and is being used in laboratory experiments to determine if this type of pathogen intervention strategy can also be used in the swine industry to aid in the reduction of enteric pathogen colonization in the swine gut.